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Qualitative Analysis of MDR-reversing Anastasia Black (Russian Black Sweet Pepper, *Capsicum annuum*, Solanaceae) Extracts and Fractions by HPLC and LC-MS-MS Methods

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Abstract. *In earlier experiments, the MDR (multidrug resistance)-reversal activities of Anastasia Black (Russian black sweet pepper) extracts had been analysed. Recently, the most effective MDR reversing extracts and fractions have been separated by HPLC (high-performance liquid chromatography, for carotenoids) and LC-MS-MS (HPLC combined with mass spectrometry, for phenolic compounds) methods. As a result of the analytical studies, the following flavonoids had been identified: feruloyl glucopyranoside, quercetin rhamnopyranoside glucopyranoside, luteolin glucopyranoside arabinopyranoside, apigenin glucopyranoside arabinopyranoside, quercetin rhamnopyranoside, luteolin arabinopyranoside diglucopy-ranoside, hesperidine and luteolin glucuronide. According to the literature, the aglycones of these phenolic compounds exhibit MDR-reversal activity in vitro, and the connection between the phenolic content of Anastasia Black and MDR-reversal action was therefore studied by different analytical methods. The results of this study revealed that the identified flavonoids of Anastasia Black may be only partially responsible for the modulation of the MDR of mouse lymphoma cells. Other lipophilic compounds, most probably carotenoids, present in Russian black sweet pepper may act as inhibitors of MDR reversal.*

Phytochemical or natural components in fruits and vegetables can modulate and enhance certain human physiological functions. The daily diet is related to the prevention of many chronic diseases, such as hypertension, diabetes or cancer, due to the presence of antimutagenic and antitumor agents. Different variants of sweet peppers are popular components of the daily diet in some countries and are rich in vitamins, carotenoids and polyphenols. These bioactive compounds have been proved to help protect aginst different chronic diseases, such as cancers and cardiovascular diseases as a consequence of their antioxidant, antimutagenic and anticarcinogenic activities (1-3). Numerous epidemiological studies have demonstrated that carotenoids may be responsible for the beneficial effects associated with the intake of carotenoid-containing vegetables (4-8). β-Carotene, α-carotene, lycopene and xanthophylls such as lutein exert significant cancer chemopreventive effects (9). The mechanisms of action of these plant-derived compounds have been examined by various methods. From recent studies it has emerged that some of the carotenoids and flavonoids have similar characteristics, such as their ability to quench free radicals or exert antioxidant activity. Nevertheless, their effects are more complex, the compounds exerting an antiproliferative action on tumor cell lines, suppressing oncogene expression, inhibiting angiogenesis and inducing differentiation in precancerous conditions (9-14).

The biological activities of two varieties of Anastasia sweet peppers (Anastasia Green and Anastasia Red, *Capsicum annuum* L. Var. *angulosum* Mill., Solanaceae) have been investigated. The dried powders of these Anastasia peppers were extracted and fractionated successively with four different eluents (hexane, acetone, methanol and 70% methanol). The extracts and fractions were examined as regards their MDR (multidrug resistance)-reversal effects with the rhodamine exclusion test on L5178 human MDR gene-transfected mouse lymphoma cells. These former

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results showed that the extracts and fractions eluted by apolar eluents were more effective than polar compounds as MDR-reversal agents (15, 16).

Anastasia Black (Russian black sweet pepper, *Capsicum annuum* L. Var. *angulosum* Mill., Solanaceae) is the most recently bred variety of Anastasia peppers and a functional food candidate. The dried powder of Anastasia Black fruit was extracted and fractionated by the use of different eluents successively. In this study, the extracts and fractions of this Russian black sweet pepper were examined for their MDR-reversal activity on L5178 human MDR gene-transfected mouse lymphoma cells and the results were compared with the MDR-modifying activities of the previously studied Anastasia variants. The extracts and fractions were analysed by HPLC (high-performance liquid chromatography) and LC-MS-MS (liquid chromatography coupled with mass spectrometry in tandem mode).

The pepper extracts and fractions are mixtures of many components with different polarities. The aim of the study was to identify the possible carotenoid and phenolic components of Anastasia Black samples which may be responsible for its biological activities. A comparison of the identified components in Russian black sweet pepper with previously described carotenoid and phenolic components with biological effects will provide information on this infrequent pepper species and will possibly serve as a basis for the discovery of new potent anticancer compounds and MDR modifiers. Recognition of the composition of the effective samples may facilitate the choice of previously unexamined components for further biological testing.

Materials and Methods

Plant material. The dried powder of Anastasia black, a variety of sweet pepper which is the unripe fruit of *Capsicum annuum* L. var. *angulosum* Mill. (Solanaceae), was cultivated in a heated greenhouse in the suburbs of Moscow and/or St. Petersburg, Russia, and was kindly donated by Field Co. Ltd.

Chemicals. The following chemicals were employed: RPMI1640 medium, Dulbecco's modified Eagle's medium (DMEM) (Gibco BRL, Grand Island, NY, USA), McCoy's 5A medium (Gibco BRL, Grand Island, NY, USA); fotal bovine serum (FBS) for RPMI medium (JRH Bioscience, Lenexa, KS, USA); horse serum for McCoy's 5A medium (Gibco BRL, Grand Island, NY, USA), rhodamine 123 (R123, Sigma Chem. Co., St. Louis, MO, USA), verapamil (Sigma Chem. Co., St. Louis, MO, USA), HPLC-grade methanol, acetonitrile and water (Merck & Co., Inc., Darmstadt, Germany), formic acid, lutein and β-carotene standards (Sigma, Milano, Italy).

Extraction procedure. Dry powdered Anastasia Black (400 g) was successively extracted with hexane, acetone, methanol (MeOH) and 70% MeOH at room temperature (Figure 1). After evaporation of the solvent *in vacuo*, the hexane extract **[H0]** yielded 5.75 g, the acetone extract **[A0]** 7.27 g, the MeOH extract **[M0]** 69.3 g and the 70% MeOH extract **[70M0]** 49.1 g.

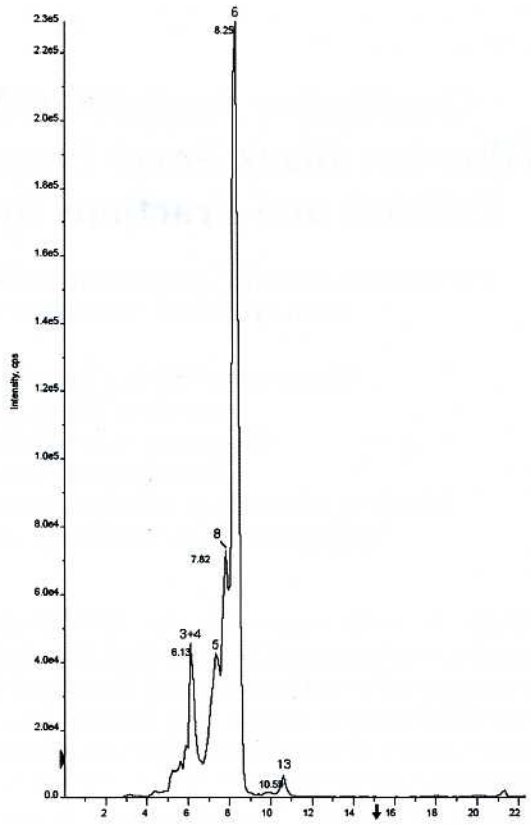

Figure 1. *LC-MS-MS analysis of Anastasia Black methanol extract [M0]: peaks and retention times of phenolic compounds [Detected phenolic compounds and retention times: quercetin rhamnopyranoside glucopyranoside (3) (6.30 min), luteolin glucopyranoside arabinopyranoside (4) (6.13 min), apigenin glucopyranoside arabinopyranoside (5) (7.33 min), quercetin rhamnopyranoside (6) (8.25 min), hesperidine (8) (7.50 min), luteolin arabinopyranoside diglucopyranoside (10), luteolin glucuronide (11) (10.59 min)].*

Initially, an aliquot of the hexane extract **[H0]** (4.5 g) was subjected to silica gel column chromatography, with step-wise elution with hexane and hexane-acetone (9:1) **[H1]** (3.03 g), hexane-acetone (4:1) **[H2]** (0.66 g), hexane-acetone (3:2) **[H3]** (0.19 g) and hexane-acetone (2:3) **[H4]** (0.06 g).

The acetone extract **[A0]** (4.8 g) was applied to a silica gel chromatographic column, and was then eluted step-wise with benzene **[A1]** (0.30 g), benzene-EtOAc (9:1) **[A2]** (0.11 g), benzene-EtOAc (4:1) **[A3]** (0.13 g), benzene-EtOAc (3:2) **[A4]** (1.37 g), benzene-EtOAc (2:3) **[A5]** (1.36 g) and EtOAc **[A6]** (1.23 g).

The MeOH extract **[M0]** (10 g) was applied to a silica gel chromatographic column and was eluted step-wise with trichloromethane ($CHCl_3$) and $CHCl_3$-MeOH (49:1) **[M1]** (0.05 g), $CHCl_3$-MeOH (24:1) **[M2]** (0.09 g), $CHCl_3$-MeOH (9:1) **[M3]** (0.43 g), $CHCl_3$-MeOH (4:1) **[M4]** (1.58 g), $CHCl_3$-MeOH (3:2) **[M5]** (0.23 g) and MeOH **[M6]** (6.48 g).

Finally, 70% MeOH extract **[70M0]** (10 g) was applied to an octadecylsilane (ODS; C18) column and was eluted step-wise with MeOH-H_2O (1:4) **[70M1]** (6.22 g), MeOH-H_2O (2:7) **[70M2]** (1.36 g), MeOH-H_2O (1:3) **[70M3]** (0.21 g), MeOH-H_2O (1:2)

[70M4] (0.24 g), MeOH-H_2O (1:1) **[70M5]** (0.49 g), MeOH-H_2O (2:1) **[70M6]** (0.41 g) and MeOH **[70M7]** (0.93 g).

Reversal of MDR of tumour cells. Assay for MDR reversal. The L5178 mouse T-cell lymphoma cell line was transfected with the human *MDR1/A* (multidrug-resistant) gene. MDR1-expressing cell lines were selected by culturing the infected cells with 60 ng/ml colchicine to maintain the expression of the MDR1 phenotype. The L5178 MDR1 T cell and parent cell lines were grown in McCoy's 5A medium with 10% heat-inactivated horse serum. The cells were adjusted to a density of $2x10^6$/mL and were resuspended in serum-free McCoy's 5A medium; 0.5 ml aliquots of the cells were distributed into Eppendorf centrifuge tubes. Then 2.0 and 20.0 µl of 1 mg/mL of the tested fractions were added and the mixtures were incubated for 10 min at room temperature. Ten microlitres of R123 indicator was added and the incubation was continued for an additional 20 min at 37°C. After washing twice and re-suspending in 0.5 ml phosphate-buffered saline (PBS), the fluorescence of the cell population was measured by flow cytometry, using a Beckton Dickinson FACScan instrument (cell sorter). Verapamil was used as positive control in the R123 accumulation tests. The R123 accumulation was calculated from the fluorescence intensity of the samples. The untreated mean fluorescence activity as a percentage of the control was calculated for the parental and MDR1 cell lines and was compared with the fluorescence of treated cells. The MDR1-reversal activity was calculated *via* the following equation:

$$\text{Fluorescence activity ratio} = \frac{MDR_{treated} / MDR_{control}}{\textit{parental treated/ parental control}}$$

LC-MS-MS method for detection of phenolic compounds. The LC-MS-MS analyses of the phenolic compounds present in the sweet pepper extracts were performed on an API 3000 triple quadrupole mass spectrometer (Applied Biosystems, Canada) equipped with a TurboIonspray source in negative mode. The operating parameters were as follows: capillary voltage (IS) -4500 V; declustering potential (DP) -60 V; heating to 400 °C. Chromatographic separation was performed on a Prodigy ODS3 100 Å column (250x4.6 mm, particle size 5 µm) (Phenomenex, CA, USA).

The eluents were the following: (A) water-0.1% formic acid; (B) CH_3CN-MeOH 60:40 (v/v). The gradient program was as follows: 30-40% B (3 min), 40-45% B (10 min), 45-50% B (2 min), 50-55% B (3 min), 55-60% B (4 min), 60-80% B (3 min), 80-90% B (2 min) and 90-30% B (3 min), at a constant flow of 0.8 mL/min. The LC flow was split and 0.2 ml/min was sent to the mass spectrometer. The injection volume was 20 µl.

The following components were examined (Table I): feruloyl glucopyranoside (**1**), sinapoyl glucopyranoside (**2**), quercetin rhamnopyranoside glucopyranoside (**3**), luteolin glucopyranoside arabinopyranoside (**4**), apigenin glucopyranoside arabinopyranoside (**5**), quercetin rhamnopyranoside (**6**), luteolin-(furanosyl-glucopyranosyl-malonyl)-glucopyranoside (**7**), hesperidine (**8**), feruyl alcohol-(methylhydroxypropionyl)-glucopyranoside (**9**), luteolin arabinopyranoside diglucopyranoside (**10**), luteolin glucuronide (**11**), ferulic acid (**12**), sinapinic acid (**13**), quercetin (**14**), luteolin (**15**) and apigenin (**16**). Table I lists the precursor ion, the MS/MS fragments and the optimum collision energy (CE) for each compound. Phenolic compounds were identified by MRM (Multiple Reaction Monitoring) and IDA (Information Dependent Acquisition) analyses based on the literature data (17).

Table I. *LC-MS-MS characteristics of several phenols found in extracts of Anastasia Black.*

Compound	Peak no.	Precursor ion $[MH]^-$ *m/z*	MS/MS ions	CE
Feruloyl glucopyranoside	**1**	355	193	–35
			134	–25
Sinapoyl glucopyranoside	**2**	385	223	–35
			208	–35
Quercetin rhamnopyranoside glucopyranoside	**3**	609	301	–49
			447	–35
Luteolin glucopyranoside arabinopyranoside	**4**	579	285	–45
			132.9	–44
Apigenin glucopyranoside arabinopyranoside	**5**	563	269	–35
			401	–35
Quercetin rhamnopyranoside	**6**	447	301	–32
			271	–45
Luteolin-(furanosyl-glucopyranosyl-malonyl)-glucopyranoside	**7**	827.5	285	–45
Hesperidine	**8**	609	315	–45
			301	–45
Feruyl alcohol-(methylhydroxypropionyl)-glucopyranoside	**9**	427.4	265.4	–35
Luteolin arabinopyranoside diglucopyranoside	**10**	741	579	–50
			285	–50
Luteolin glucuronide	**11**	461	285	–40
Ferulic acid	**12**	192.7	133.9	–25
			178	–15
Sinapinic acid	**13**	222.7	207.8	–20
			164	–19
Quercetin	**14**	300.7	150.9	–28
			179.1	–24
Luteolin	**15**	284.9	132.9	–44
			151	–32
Apigenin	**16**	269	151	–35

CE: collision energy.

HPLC method for detection of carotenoid compounds. The carotenoid components of the Anastasia Black extracts and fractions were separated on an HPLC system, involving a Devosil RP Aqueous C30 column (250x4.6 mm, particle size 5 µm). The eluents were the following: (A) 0.004% ammonium acetate (solvent: MeOH) and (B) *tert*-butylmethyl ether. The gradient program was as follows: 85-70% B (5 min), 70-60% B (5 min), 60-45% B (5 min), 45% B isocratic (20 min), 45-20% B (5 min), and 20-85% B (5 min), at a constant flow of 1 ml/min. The detection wavelength was 453 nm and the injected volume was 20 µl. Lutein and β-carotene were injected as positive controls.

Results

Enhancement of the expression of *MDR1/A* in the L5178 mouse T-cell lymphoma cell line resulted in MDR, as

reflected by the reduced intracellular accumulation of R123, while the addition of verapamil reversed the MDR1, as reflected by the increase in R123 accumulation (6.13-fold increase) (Table II). One extract and four column fractions displayed higher MDR1-reversal activities as compared with the MDR1 control (fluorescence activity ratio=1) or verapamil control (fluorescence activity ratio=6.13). The 4 μg/mL hexane fraction **[H4]** (fluorescence activity ratio=14.72) had the highest MDR1 reversal activity, followed by **[H2]** (fluorescence activity ratio=14.72) and **[H3]** (fluorescence activity ratio=7.24). The 40 μg/ml hexane fraction corresponded to the highest increase in R123 accumulation **[H4]** (fluorescence activity ratio=81.74), followed by **[H2]** (fluorescence activity ratio=57.95), **[H0]** (fluorescence activity ratio=26.1), **[A6]** (fluorescence activity ratio=21.68) and **[H3]** (fluorescence activity ratio=20.94).

Some acetone fractions exhibited relatively high fluorescence activity ratios: **[A2]** ($FR_{4\ \mu g/ml}$ =22.94), **[A4]** ($FR_{40\ \mu g/mL}$ =12.65), **[A5]** ($FR_{40\ \mu g/mL}$ =13.89). The above results demonstrate that the effect of MDR1 reversal correlates with the polarity and the concentration of the Anastasia Black extracts.

The measured FSCs (forward light scatter) and SSCs (side light scatter) of the Anastasia Black-treated cells did not differ significantly from those of the parent or MDR1 control cells. Thus, it can be stated that the samples had only slight or no cytotoxic activity (Table II).

Samples **[A0]**, **[M0-M6]** and **[70M0-70M7]** were analysed by LC-MS-MS for phenolic compounds. Sixteen different phenols were investigated, including glycosylated molecules and their aglycones (17) (Table III). The results of the qualitative analysis showed that 8 out of the total of 16 phenols generated defined peaks. Six of these compounds had been reported in the literature, while luteolin glucuronide and luteolin arabinopyranoside diglucopyranoside were identified for the first time by IDA (information dependent acquisition) experiments and were then examined in multiple reaction monitoring (MRM) analyses. The extract **[M0]** contained the most of the compounds: seven peaks were noted for the following compounds (with their retention times): quercetin rhamnopyranoside glucopyranoside (**3**) (6.30 min), luteolin glucopyranoside arabinopyranoside (**4**) (6.13 min), apigenin glucopyranoside arabinopyranoside (**5**) (7.33 min), quercetin rhamnopyranoside (**6**) (8.25 min), hesperidine (**8**) (7.50 min), luteolin arabinopyranoside diglucopyranoside (**10**), and luteolin glucoronide (**11**) (10.59 min). The data are illustrated in Figure 1. Fractions **[M2]**, **[M5]** and **[M6]** of the **[M0]** extract furnished the peaks of compounds **3**, **4**, **6** and **8**. The more polar the fractions were, the more polyphenols were detected. Five peaks were noted in extract [A0]: compounds **3**, **4**, **6**, **8** and **11**. In this case, the retention time of hesperidine (**8**) was 7.36 min. In extract **[70M0]**, four different peaks appeared, those of compounds **1**, **3**, **4** and **6**. The LC-MS-MS analysis revealed that the components were eluted in the order of increasing polarity in the fractions of this extract. The peak of feruloyl glucopyranoside (**1**) was noted in fraction **[70M4]**, quercetin rhamnopyranoside glucopyranoside (**3**) and luteolin glucopyranoside arabinopyranoside (**4**) were detected in fraction **[70M5]**. In addition to compounds **3** and **4**, quercetin rhamnopyranoside (**6**) was present in fraction **[70M6]**. The most marked peaks were due to quercetin rhamnopyranoside (**6**), the most characteristic flavonoid component of the sweet pepper samples studied.

HPLC of samples **[H0]** and **[A0-A6]** allowed for the identification of the carotenoid components through comparison with the peaks of lutein and β-carotene as

Table II. *MDR-reversal activity of Anastasia Black extracts and fractions.*

Sample	FSC	SSC	Fluorescence activity ratio
PAR control	534.83	127.47	-
MDR control	584.65	144.76	1
MDR +R123(mean)	588.09	147.21	-
25% DMSO control	535.24	130.56	0.92
Verapamil 10 μg/mL (positive control)	620.20	156.09	6.13

Anastasia Black	Concentration (μg/ml)		Concentration (μg/ml)		Concentration (μg/ml)	
	4	40	4	40	4	40
H0	497.21	613.16	149.04	177.33	5.52	**26.10**
H1	571.64	577.30	148.15	140.53	3.73	4.03
H2	579.37	572.08	144.99	158.05	**12.45**	**57.95**
H3	573.74	577.81	139.05	146.25	7.24	**20.94**
H4	578.34	581.55	139.27	150.82	**14.72**	**81.74**
A0	578.84	585.76	137.58	143.40	2.84	7.05
A1	588.46	582.66	140.29	143.85	1.26	1.44
A2	577.97	587.32	154.78	155.81	**22.94**	15.83
A3	577.11	590.23	137.43	144.05	3.72	8.08
A4	608.62	626.99	193.35	182.67	2.22	12.65
A5	625.77	636.51	169.66	172.49	6.72	13.89
A6	619.82	609.02	164.29	157.74	2.46	**21.68**
M0	603.84	608.37	154.32	154.90	0.66	0.64
M1	584.70	597.54	145.94	156.06	0.70	1.65
M2	580.01	549.51	144.16	138.18	2.08	2.49
M3	543.14	540.68	136.66	136.40	4.25	7.82
M4	534.99	530.06	129.78	128.82	0.47	0.46
M5	525.13	514.85	128.32	127.99	0.46	0.45
M6	518.02	517.27	127.26	127.40	0.44	0.42
70M0	595.88	566.05	139.98	139.77	0.79	0.54
70M1	563.79	555.99	142.45	137.96	0.72	0.46
70M2	549.99	547.96	135.34	135.62	0.46	0.41
70M3	543.17	541.60	133.04	135.41	0.42	0.33
70M4	541.98	534.05	129.18	130.62	0.36	0.33
70M5	535.15	528.76	127.93	123.61	0.32	0.31
70M6	526.09	524.07	123.66	124.49	0.28	0.33
70M7	518.30	520.50	121.18	120.88	0.32	0.42

FSC: forward light scatter.
SSC: side light scatter.

Table III. *Qualitative analysis of Anastasia Black extracts and fractions by LC-MS-MS.*

Samples	Flavonoid compounds[1]															
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
H0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A0	-	-	+	+	-	+	-	+	-	-	+	-	-	-	-	-
M0	-	-	+	+	+	+	-	+	-	+	+	-	-	-	-	-
M1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M2	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
M3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M5	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-
M6	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-
70M0	+	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-
70M1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
70M2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
70M3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
70M4	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
70M5	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
70M6	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-
70M7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[1]Flavonoid components: feruloyl glucopyranoside **(1)**, sinapoyl glucopyranoside **(2)**, quercetin rhamnopyranoside glucopyranoside **(3)**, luteolin glucopyranoside arabinopyranoside **(4)**, apigenin glucopyranoside arabinopyranoside **(5)**, quercetin rhamnopyranoside **(6)**, luteolin-(furanosyl-glucopyranosyl-malonyl)-glucopyranoside **(7)**, hesperidine **(8)**, feruyl alcohol-(methylhydroxypropionyl)-glucopyranoside **(9)**, luteolin arabinopyranoside diglucopyranoside **(10)**, luteolin glucuronide **(11)**, ferulic acid **(12)**, sinapinic acid **(13)**, quercetin **(14)**, luteolin **(15)** and apigenin **(16)**.

standards. In extract **[H0]**, carotenoids could not be detected and therefore the fractions **[H1-H4]** were not analysed. A peak in extract **[A0]** was assigned to lutein, but this peak was not detectable in the fractions. Most components of **[A0]** appeared in the fraction **[A4]** during benzene-EtOAc elution, but no peak in that fraction corresponded to lutein and other peaks were not definite.

Discussion

MDR in tumour cells is a significant problem in the chemotherapy of many cancers. MDR often originates from elevated expressions of particular proteins, such as cell-membrane transporters, which can result in an increased efflux of the chemotherapeutic agents from the cells, thereby lowering their intracellular concentration (18).

The results of the present study demonstrate that Anastasia Black extracts and their fractions reversed the MDR of mouse T-lymphoma cells. P-glycoproten (P-gp), which belongs in the ATP-binding cassette (ABC) transporter family, reduces the cytotoxic activity of anticancer agents in tumor cells, such as etoposide, vinblastin, mitomycin C, doxorubicin and actinomycin D, by pumping them out of the cytoplasm of the cells. In our study, R123 was a model substance of these antitumor agents. The results indicate that samples extracted or eluted with an apolar solvent were more efficient than the polar ones and thus the apolar carotenoids, vitamins and polyphenols of Anastasia Black are probably responsible for the MDR1-reversal activity.

Sixteen different phenolic compounds were studied by LC-MS-MS in Anastasia Black extracts and fractions. The investigated compounds were identified previously in different plant species. Compounds **1-7** and **9** previously isolated from hot pepper (*Capsicum annuum* L. var. Bronowiczka Ostra) and the structures were determined. Quercetin rhamnopyranoside was found for the first time in pepper species in a study by Materska *et al.* (17). This glycosylated flavonoid occurs in many plants and its antioxidant ability has been demonstrated (19-21). The aglycone quercetin is a potent biomolecule due to its antioxidant activity. *In vitro* studies on cells and pure DNA have indicated the possible mechanisms of action. Quercetin can protect against mitomycin C-induced DNA strand breakage and exhibits antiproliferative activity (22, 23). There is an emerging view that flavonoids not only act as conventional hydrogen-donating antioxidants, but may exert modulatory action in cells *via* the protein kinase and lipid kinase signalling pathways (24).

The MDR-reversal effects were detected in those Anastasia Black extracts and fractions which were eluted by less polar solvents. Lipophilic components, e.g., carotenoids, may therefore be responsible for the P-gp inhibitory effect. Lutein- and β-carotene-based HPLC analisys of samples **[H0]** and **[A0-A6]** was performed in order to investigate the carotenoid content of Anastasia Black. The isolated lutein and β-carotene

had previously been investigated for their MDR-reversal activities on human MDR1/A gene-transfected mouse lymphoma cells, MDA MB 231 (HTB-26) human breast cancer cells and Colo 320 human colon cancer cells *in vitro*. Lutein increased the R123 accumulation of MDR mouse lymphoma cells and colon cancer cells, but β-carotene displayed no modulating activity on the drug sensitivity of mouse lymphoma or breast cancer cells (11, 25). The mechanism of action in mouse lymphoma cells is presumed to be charge-transfer complex formation with P-gp (11).

The sweet pepper samples eluted with less polar eluents exerted higher bioactivity due to their lipophilic components. The MDR-reversing carotenoids were detected in very small amounts in these samples and it may therefore be assumed that other lipophilic compounds are responsible for the MDR-reversal activity. Some of the isolated carotenoids from Anastasia Black pepper are able to act as resistance modifiers.

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